STUDIES ON THE CLASSIFICATION OF THE COLON GROUP *

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The first biologic division of the colon group was made by Smith[1] in 1895. He divided all lactose-fermenting, non-liquefying organisms into groups according to their fermentation of saccharose. This classification was corroborated by many other workers and has found general acceptance. In 1905 MacConkey[2] suggested a further subdivision of these two groups based on the power of the organisms to ferment dulcite. Not satisfied that this classification distinguished sufficiently well the different types of organisms belonging to the colon groups, he later[3] proposed a further subdivision based on a number of biochemical differences, such as fermentation of certain other sugars, indol production, Voges Proskauer reaction, etc. Jackson,[4] following MacConkey's lead, separated the group into sixteen distinct types.

The first attempt at a biologic classification based on the biometric principles, as outlined by the Winslows in 1908,[5] was made by Howe in 1912.[6] He worked with 540 strains of lactose-fermenting, non-liquefying bacteria and concluded that motility, quantity of gas, fermentation of mannite, dulcite and starch do not correlate with any other properties, and hence are of little value in classifying this group. He confirms the basic division made by Smith and claims that dextrose, lactose, saccharose and raffinose constitute a true "metabolic gradient," and that fermentation of any one sugar implies fermentation of those preceding it in the series.

The work presented in this paper consists of a study of 80 organisms generally included in the colon group. Among the cultures studied were *B. coli communis, coli communior, acidi-lactici, coscoroba, aerogenes, capsulatus, pneumoniae, viscosus, cloacae, proteus* and *enteritidis*. These were all laboratory strains, at least 2 years old,

* Received for publication March 12, 1914.

1. *Centralbl. f. Bakteriol.*, 1895, 18, p. 494.
2. *Jour. Hyg., Cambridge,* 1905, 5, p. 333.
3. *Ibid.,* 1909, 9, p. 86.
4. *Jour. Infect. Dis.,* 1911, 8, p. 241.
5. Systematic Relationship of the Coccaceal, 1908.
6. *Science,* 1912, 35, p. 225.

isolated originally from a variety of sources: polluted water, feces, urine, the animal body, etc. The following tests were applied: morphology, Gram reaction; fermentation of dextrose, lactose, saccharose, raffinose, glycerin, salicin, mannite, dulcite and inulin; action on milk and gelatin; reduction of nitrates; indol production, and the Voges and Proskauer reaction.

All the organisms studied were small uniformly staining rods (average 1.5 μ. by 0.7 μ.), reacting negatively to Gram, capable of fermenting one or more of the sugars (a few exceptions) with the production of either acid and gas, or acid alone. Of the 80 strains, 52 produced gas in both dextrose and lactose, 20 produced gas in dextrose and not in lactose, and 8 produced gas in neither. Of these 8, 5 produced acid but no gas in both of the sugars and will be included among the lactose fermenters, while 1 fermented dextrose and not lactose, and 2 failed to ferment at all. One of the last 2 came to us as *Bacillus mirabilis* and neither fermented nor liquefied and probably ought not to be classed with the proteus group at all; the other was sent to us as *Bacillus pneumoniae* and had either lost its fermentative properties or had been overgrown by another organism; no capsule could be observed. The dextrose fermenter is a cholera bacillus and was included with 2 others for comparison; the other 2 fermented with gas production, while this one did not ferment. It is also culturally different from the other 2, and really belongs to the septicemiae group. These 3 organisms will be excluded in the discussion of the results.

The fermentation tests were all conducted in standard meat infusion, sugar-free broth, to which 1 per cent. of the desired sugar was added. The cultures were incubated from four to five days at 37 C., duplicate 5 c.c. samples were titrated with phenolphthalein as an indicator, and the average of the two results recorded. Controls were titrated but the initial acidity was not subtracted, as it was found that between certain limits the final acidity was a very constant quantity independent of the degree of initial acidity. This was shown to be the case by the following experiment: A few organisms selected at random from among those studied were inoculated into sugar broths of different acidities, varying from 0.5 to 3 per cent. normal acid, and titrated at various intervals. Five organisms and four of the test substances were employed. In all cases the acidity rose to a certain point which was quite constant for each organism in each sugar. The time required to reach the maximum acidity varied to some extent, of course, for the different organisms, but in most cases it was obtained in three, and rarely later than in four days. In the broth with the higher initial acidity there was a slight preliminary retardation, but the final result was about the same. The facts are summarized in Table 1.

A comparison between the acid and gas production in meat-infusion dextrose broth and a synthetic dextrose broth showed a decided varia-

tion in the latter. Many of the actively fermenting organisms in dextrose broth failed to ferment or fermented only feebly in the synthetic broth. This corresponds with Miss Broadhurst,[7] who reported recently of the marked difference of acid production by streptococci in meat extract and meat infusion broth, respectively. Both these findings point out the importance of studying these organisms in a more or less uniform but always favorable environment. Under other conditions the results are not sufficiently constant to indicate the real relationship of the organisms studied.

TABLE 1

EFFECT OF INITIAL ACIDITY ON THE TOTAL FINAL ACIDITY

Sugar	Initial Acidity	Organisms Used				
		37	44	53	60	72
		Maximum Acidities				
Dextrose	0.6	3.3	5.1	3.8	3.2	3.8
	1.7	3.4	4.7	3.2	3.8	3.7
	1.9	3.8	4.8	4.0	4.0	3.7
	2.6	4.6	4.9	3.9	4.2	4.2
Lactose	0.6	3.5	4.5	5.3	5.2	5.2
	1.6	3.8	4.5	5.4	5.7	5.6
	2.0	3.7	4.5	5.0	5.5	5.1
	2.5	4.1	4.4	5.1	5.0	5.1
Saccharose	0.2	4.6	3.1	4.0	4.6	4.9
	1.5	4.7	3.6	4.7	5.2	5.1
	2.0	4.7	3.4	4.6	4.6	5.0
	2.4	5.0	3.4	4.6	4.7	4.8
Glycerin	0.2	3.1	2.1	3.6	4.0	2.5
	1.5	3.4	2.2	3.0	3.6	3.1
	1.8	3.6	2.0	3.3	3.2	3.6
	2.4	3.5	2.3	3.4	3.4	3.1

Indol was tested for in peptone broth after four days' incubation at 37 C. The Ehrlich test was found, after a careful comparative study,[8] to be more reliable than the Salkowsky, and was used throughout. Nitrites were tested for in a nitrate-peptone broth in the usual way. Gelatin liquefaction was determined in small tubes 1 cm. in diameter, the depth of liquefaction after thirty days at 20 C. being recorded.

The fermentation reactions of the group are summarized in Figures 1 and 2. The most striking points brought out by these charts are the importance of quantitative titration of the acid produced and the unreliability of gas production, as ordinarily tested for as a distinguishing character. The work of Keyes and Gillespie[9] shows a marked con-

7. *Jour. Infect. Dis.*, 1913, 13, p. 404.
8. *Ibid.*, 1914, 14, p. 81.
9. *Jour. Biol. Chem.*, 1912, 13, p. 305.

stancy in the gas ratio when the test is carried out under carefully controlled conditions in a sealed tube. In the open tube, however, where solution and diffusion of the gases are constantly taking place, and where the physical factors of pressure and temperature exert such a decided influence on the phase equilibrium, there is no such constancy observed. It is doubtful, however, whether it is necessary to

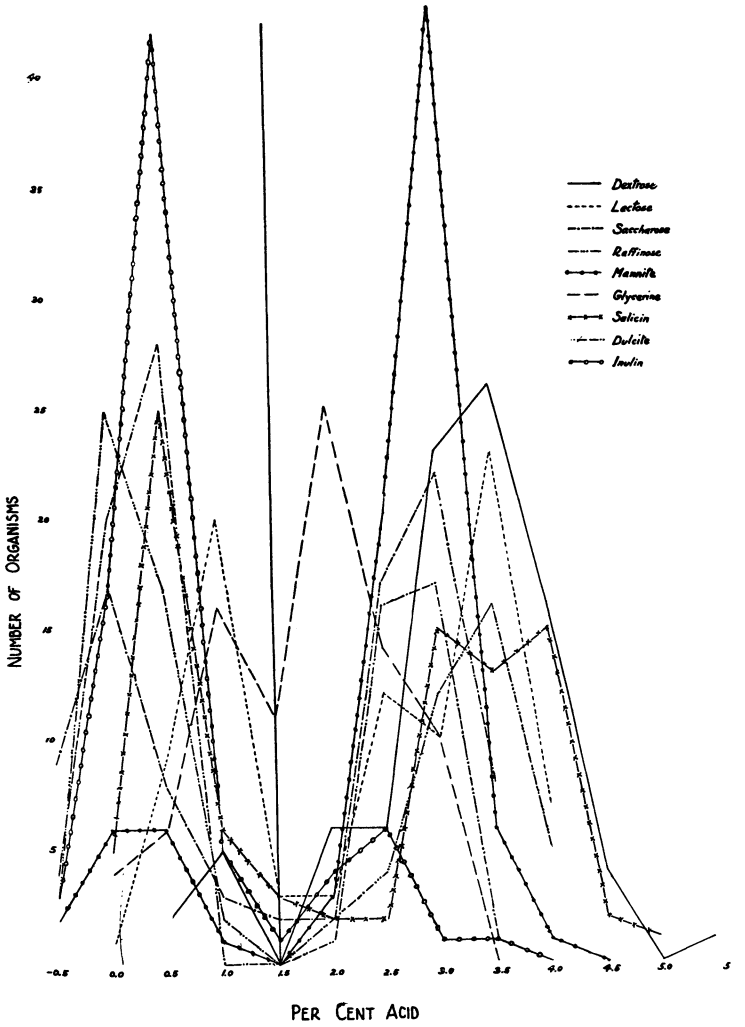

FIG. 1.—Acid production in various sugars by members of the colon-typhoid group. Note the sharp break between fermenters and non-fermenters at 1.5 per cent normal acid.

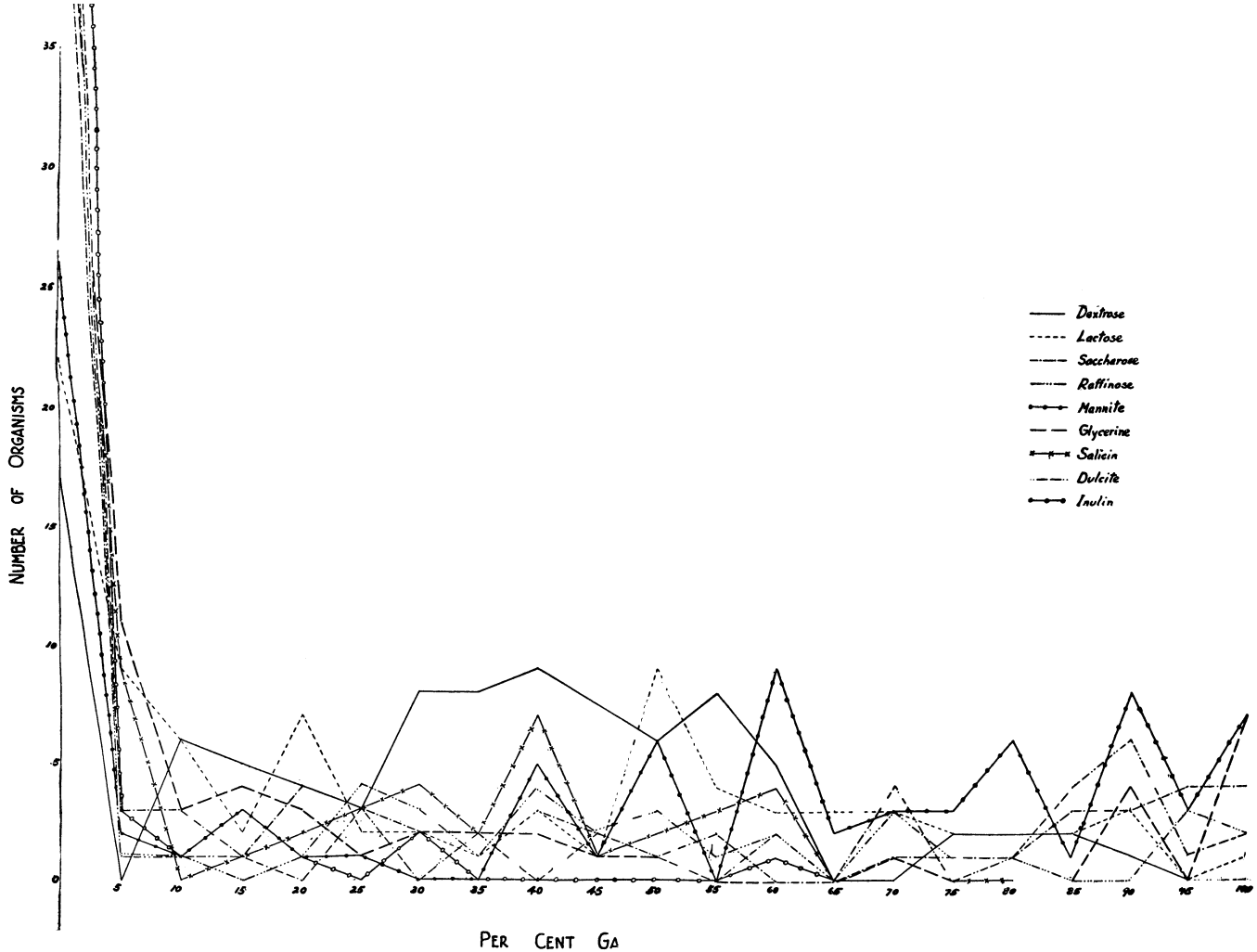

FIG. 2.—Gas production in various sugars by bacteria of the colon-typhoid group. Note the absence of a sharp point of division.

employ the elaborate method of Keyes and Gillespie for testing gas ratios and gas quantities, when acid production gives such clear-cut differentiation when the acid is titrated quantitatively. Many organisms are capable of producing just enough acid to turn blue litmus red, but should not be counted as acid producers. As shown by Figure 1, the dividing line between acid and non-acid producers is very definite and generally falls at 1.5 per cent. normal acid. The neutral point for litmus is usually between 1.0 and 1.2 per cent. normal acid, depending on the grade. Therefore, while the litmus test may often be misleading, this is not the case when quantitative titrations are made with phenolphthalein as an indicator. The quantity of gas produced, on the other hand, fluctuates very much and gives no such regular curve as does acid production (Fig. 2). Many organisms too, while typical in every other respect, have apparently either partly or wholly lost their power to produce gas, judging by the names under which they were sent to us. The members of the proteus group, on the other hand, produced from 10 to 20 per cent. gas in lactose broth, though at no time did they produce more than 1.0 per cent. normal acid.

The extent to which the various sugars used are fermented (considering both acid, and acid and gas as positive) is of interest. Table 2 shows the order of availability of these substances for the entire group and for the lactose-fermenters only.

TABLE 2

SHOWING THE NUMBER OF ORGANISMS ATTACKING DIFFERENT SUGARS

Sugar Fermented	Dextrose	Mannite	Lactose	Glycerin	Saccharose	Salicin	Raffinose	Dulcite	Inulin
All organisms studied	77	71	57	51	49	45	41	36	11
Lactose-fermenters only	57	57	57	48	41	39	38	28	9

The frequency with which the various sugars are fermented agrees in the main with the results obtained by Howe. Dextrose is fermented by all the bacteria tested. Next in order is mannite, followed by lactose. The first real division, as already well established, occurs in the fermentation of lactose. The colon-aerogenes-cloacae group is lactose-positive and the proteus-enteritidis group is lactose-negative.

Mannite seems of comparatively slight value for differential purposes, (1) because it is attacked by so large a proportion of the dextrose-fermenting strains (71 out of 77), and, (2) because it is not correlated with other properties,

the six non-fermenting strains belonging to the proteus group, with the exception of one which belongs to the enteritidis.

Considering for the present only the lactose-fermenting group, we note that glycerin and saccharose are the next substances most frequently attacked. The forms which ferment glycerin and not saccharose are scattered among many sub-groups and do not apparently represent a series of related forms. Saccharose, as Smith[10] and Howe[11] have pointed out, seems to correspond to the first broad sub-division of the lactose-fermenting colon bacilli. In my own series, saccharose divides the group into two parts of approximately 70 per cent. and 30 per cent., respectively. Howe finds that saccharose divides his 540 lactose-fermenting organisms into groups of approximately 60 per cent. and 40 per cent., respectively. The agreement between my comparatively few, old stock, strains and Howe's freshly isolated bacilli is close enough to indicate that this is a uniform phenomenon.

Raffinose correlates so closely with the fermentation of saccharose that its importance as a classificatory substance is still debatable. Of the 57 lactose-fermenting organisms studied, 65 per cent. are saccharose-positive, raffinose-positive; 27 per cent. saccharose-negative., raffinose-negative; 5 per cent. saccharose-positive, raffinose-negative, and only 3 per cent. saccharose-negative, raffinose-positive. Howe gets 53 per cent., 41 per cent., 5 per cent. and 1 per cent., respectively. It is doubtful whether the 5 per cent. of aberrant strains deserve definite specific recognition. Winslow (1907)[12] has also found that fermentation of raffinose generally follows very closely that of saccharose. It is very likely that the same processes of hydrolysis and subsequent fermentation takes place in both cases. The sugars are very much alike chemicallly, in that neither reduces, and both yield fructose and glucose on hydrolysis.

Of the remaining sugars, inulin is fermented by only a few of the organisms (9), and of these, 8 belong to the aërogenes group. It does not, however, correlate with indol production or the Voges and Proskauer reaction, and does not appear to be of classificatory importance.

The substances left to be considered are salicin and dulcite. Of the two, dulcite is at present widely used to further differentiate the saccharose groups. It remains to be seen from an analysis of the results obtained whether or not it really correlates with other characters in such a way as to give a biological subdivision. Howe[13] in the abstract of his results claims that dulcite does not correlate with any other character.

That the saccharose-positive group consists of more than one species is evident when one compares the saccharose-fermenting organisms now generally known as *Bacillus communior* and *Bacillus aërogenes,* respectively. The question is: Is the dulcite division biologically sound, and if not, what test or tests would give a better subdivision? Of the other tests performed, coagulation of milk and nitrate reduction are characteristic of the entire group, and hence of little classificatory

10. *Centralbl. f. Bakteriol.,* 1895, 18, p. 494.
11. *Science,* 1912, 35, p. 225.
12. *Ibid.,* 1907, 26, p. 797.
13. *Ibid.,* 1912, 35, p. 225.

value. The Voges and Proskauer reaction is supposed to be given by the aërogenes-cloacae group only, and indol production is generally considered typical for the communis-communior group. Salicin, though found by Hilliard[14] to be an important test substance for the streptococci, has not been employed for the classification of this group. Table 3 gives the correlation of indol production, Voges and Proskauer reaction, gelatin liquefaction and fermentation of glycerin with the dulcite-saccharose and the salicin-saccharose groups, respectively.

TABLE 3

CORRELATION OF DULCITE AND SALICIN FERMENTATION RESPECTIVELY WITH OTHER CHARACTERS

	Organism	Per Cent Indol Positive	Per Cent V and P Positive	Per Cent Glycerin Fermenters	Per Cent Gelatin Liquefiers	Per Cent Salicin Fermenters
Saccharose-positive Dulcite-positive ..	20	50	20	85	5	50
Saccharose-positive Dulcite-negative ...	20	25	70	70	15	90
Saccharose-negative Dulcite-positive ...	9	100	0	100	0	89
Saccharose-negative Dulcite-negative ...	8	63	0	88	0	38
						Per Cent Dulcite Fermenters
Saccharose-positive Salicin-positive	28	21	68	68	14	35
Saccharose-positive Salicin-negative ...	12	75	0	100	0	83
Saccharose-negative Salicin-positive ...	11	100	0	100	0	73
Saccharose-negative Salicin-negative ...	6	50	0	83	0	17

Table 3 shows that the saccharose-positive salicin-positive group corresponds closely with the saccharose-positive dulcite-negative group. The former gives, however, a more definite division, in that it includes all the bacteria which liquefy gelatin and gives the Voges and Proskauer reaction. Again, unlike the dulcite group, it is the only group which gives a large number of glycerin non-fermenters, a negative property which, as will be shown later, correlates with gelatin liquefaction and is characteristic of the cloacae group. Of the other groups, the saccharose-positive salicin-negative corresponds with the saccharose-positive dulcite-positive; 83 per cent. of the former fermenting dulcite; the saccharose-negative salicin-positive group agrees closely with the saccharose-negative dulcite-positive, and the saccharose-nega-

14. *Jour. Infect. Dis.*, 1913, 12, p. 144.

tive salicin-negative group corresponds to the saccharose-negative dulcite-negative.

Neither the saccharose-dulcite nor the saccharose-salicin groups are sharply defined. It is, however, apparent from Table 3 that for the organisms studied salicin gives a more thoroughly biological classification, in that it correlates more closely with the other properties, and is also the more available of the two substances, as shown in Table 2. A sharp division of the groups on any basis could hardly be expected when one considers the large number of intermediary forms present among all groups of bacteria, and especially prevalent in this group.

A detailed comparison of the reactions of these 4 groups is given in Tables 4 to 7, inclusive; those headed "a" represent the dulcite groups, while those headed "b" the salicin groups. The main features brought out by these tables have been summarized in Table 3. A few additional points are, however, worth mentioning. The salicin grouping, while it may include in the aërogenes group organisms which, though probably on the border line, belong rather to the communior division, it does on the other hand include all of the capsulatus and pneumoniae forms, which undoubtedly fall in the same group, if not in the same species, as does *Bacillus aërogenes*. This is not the case with the dulcite classification, as Nos. 15, 37, 62 and 64 are obviously, both culturally and morphologically, members of the aërogenes group, and yet, according to their dulcite fermentation, are classed with *Bacillus communior*. *Bacillus aërogenes* is generally decidedly different morphologically, from the other 3 species. The rods are, as a rule, shorter and thicker than the others; the growth on the agar streak is heavy, translucent and often viscid, instead of the flat almost transparent growth of *Bacillus communior,* etc., and in broth a thick pellicle is generally formed. These characters, though in themselves variable and of little differential value, are undoubtedly closely linked with the peculiar property of capsule formation manifested by this group, and while their absence does not necessarily exclude the organism from this species, as they are often lost on prolonged cultivation, their presence should certainly class them with *Bacillus aërogenes*. These points must not, of course, be pushed too far. They are merely indications of probable relationships which should be corroborated by more extensive studies with a larger number of organisms. It is also interesting to note that in the saccharose-salicin grouping practically all the forms derived from milk fall into either the aërogenes or the lactici groups

TABLE 4 (a)

SHOWING THE REACTIONS OF THE SACCHAROSE-POSITIVE DULCITE-NEGATIVE GROUP (B. AEROGENES-MACCONKEY).

No.	Fermentation of Salicin	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
3	+	+	−	−	+	B. gasoformans	Water
7	+	+	−	−	−	B. viscosus	Milk
10	−	+	−	−	+	B. of ropy milk	Milk
36	−	+	+	−	−	B. aërogenes B2	Feces
39	+	+	+	−	−	B. aërogenes A1	Urinary fistula
40	+	+	+	−	+	B. aërogenes A2	Polluted well-water
48	+	+	+	−	−	Enterococcus	
50	+	+	−	+	+	B. cloacæ	Lockport canal
51	+	+	−	+	+	B. cloacæ	Intestine of sparrow
53	+	+	−	−	+	B. capsulatus	Kral
54	+	+	+	−	−	B. pneumoniæ	
58	+	+	−	−	+	B. aërogenes	Lung
59	+	−	−	−	+	B. cloacæ	B. of A. In. 1897
60	+	+	−	−	+	Pfeiffer's b.	Original capsule bacillus of Pfeiffer
61	+	+	−	−	+	B. pneumoniæ	
65	+	+	−	−	+	B. pneumoniæ	Novy
66	+	+	+	−	+	B. aërogenes	Milk
72	+	+	−	−	+	B. capsulatus	
87	+	+	−	−	+	Paracolon b.	Milk
91	+	+	−	−	+	Paracolon b.	Milk

TABLE 4 (b)

SHOWING THE REACTIONS OF THE SACCHAROSE-POSITIVE SALICIN-POSITVE GROUP (B. AEROGENES-KLIGLER).

No.	Fermentation of Dulcite	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
1	+	−	−	−	−	B. communior (lutea)	Feces
3	−	+	−	−	+	B. gasoformans	Water
7	−	+	−	−	−	B. viscosus	Milk
10	−	+	−	−	+	B. of ropy milk	Milk
15	+	+	+	−	+	B. of ropy milk	Ropy cream
33	−	+	−	−	−	B. coli communis	Dysentery stool
37	+	+	−	−	+	B. communior A1	Cystitis
40	+	+	−	+	+	B. aërogenes A2	Polluted well-water
48	−	+	+	−	−	Enterococcus	
50	−	−	−	+	+	B. cloacæ	Lockport canal
51	−	+	−	+	+	B. cloacæ	Intestine of sparrow
53	−	+	−	−	+	B. capsulatus	Kral
54	−	+	+	−	−	B. pneumoniæ	
58	−	+	−	−	−	B. aërogenes	Lung
59	−	−	−	−	+	B. cloacæ	B. A. I. 1897
60	−	+	−	−	+	Pfeiffer's b.	Original capsule bacillus of Pfeiffer
61	−	+	−	−	+	B. pneumoniæ	
62	−	+	−	−	+	B. aërogenes	Autopsy
64	−	+	+	−	+	B. aërogenes	Kral
65	−	−	−	−	+	B. pneumoniæ	Novy
66	−	+	+	−	+	B. aërogenes	Milk
70	+	−	−	+	−	B. cloacæ	Milk
72	−	+	−	−	+	B. capsulatus	J. H. U.
87	−	+	−	−	+	Paracolon b.	Milk
90	+	+	−	−	−	Paracolon b.	Milk
91	−	+	−	−	+	Paracolon b.	Milk
88	+	+	−	+	−	Paracolon b.	Milk
89	+	−	−	−	−	Paracolon b.	Milk

TABLE 5 (a)

SHOWING THE REACTIONS OF THE SACCHAROSE-POSITIVE DULCITE-POSITIVE GROUP (B. COMMUNIOR-MACCONKEY).

No.	Fermentation of Salicin	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
1	+	−	−	−	−	B. communior (lutea)	Feces
15	+	+	+	−	+	B. of ropy milk	Ropy cream
20	−	+	+	−	−	B. communior (rubra)	Water
27	−	+	+	−	−	B. communior A2	Feces
28	−	+	+	−	−	B. coli communis	Stool
30	−	+	+	−	−	B. coli communis	
33	+	+	+	−	−	B. coli communis	Dysentery stool
37	−	+	+	−	+	B. communior A1	Cystitis
41	−	+	+	−	−	B. communior C	Feces
43	−	+	+	−	−	B. communior B	Cystitis
44	−	+	+	−	−	B. coli communis	Cystitis
45	−	+	+	−	−	B. coli communis	Water
46	−	+	+	−	−	B. coli communis	Bird feces
47	−	+	+	−	−	B. coli communis	Milk
62	+	+	−	−	+	B. aërogenes	Autopsy
64	+	+	−	−	+	B. aërogenes	Kral
70	+	+	−	+	−	B. cloacæ	Milk
88	+	−	−	−	−	Paracolon b.	Milk
89	+	+	−	−	−	Paracolon b.	Milk
90	+	−	−	−	−	Paracolon b.	Milk

TABLE 5 (b)

SACCHAROSE-POSITIVE SALICIN-NEGATIVE (B. COMMUNIOR-KLIGLER)

No.	Fermentation of Dulcite	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
20	+	+	+	−	−	B. communior	Water
27	+	+	+	−	−	B. communior A_2	Feces
28	−	+	+	−	−	B. coli communis	Stool
30	−	+	+	−	−	B. coli communis	
36	+	+	+	−	−	B. aërogenes B_2	Feces
39	+	+	+	−	−	B. aërogenes A_1	Urinary fistula
41	+	+	+	−	−	B. communior C	Feces
43	+	+	+	−	−	B. communior B	Cystitis
44	+	+	+	−	−	B. coli communis	Cystitis
45	+	+	+	−	−	B. coli communis	Water
46	+	+	+	−	−	B. coli communis	Bird feces
47	+	+	+	−	−	B. coli communis	Milk

(Tables 4 b and 7 b), while the communior and communis groups (Tables 5 b and 6 b) consist of those forms obtained from the animal body. This is not the case with the saccharose-dulcite division. Another point worth noting is that the pathogenic forms, *Bacillus columbarum* and *Bacillus cholerae,* fall in the species of *Bacillus communis.*

TABLE 6 (a)

SHOWING THE REACTIONS OF THE SACCHAROSE-NEGATIVE DULCITE-POSITIVE GROUP (B. COLI COMMUNIS-MACCONKEY)

No.	Fermentation of Salicin	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
23	+	+	+	—	—	B. columbarum	Kral
29	+	+	+	—	—	B. coli communis	Novy
31	+	+	+	—	—	B. coli communis	P. D. & Co.
32	+	+	+	—	—	B. coli communis	
35	+	+	+	—	—	B. coli communis	Feces
38	+	+	+	—	—	B. coli communis B.	Cystitis
63	+	+	+	—	—	B. coli communis	Autopsy
79	+	+	+	—	—	B. choleræ	Kral
86	+	+	+	—	—	B. choleræ	Rahn

TABLE 6 (b)

SHOWING THE REACTIONS OF THE SACCHAROSE-NEGATIVE SALICIN-POSITIVE GROUP (B. COLI COMMUNIS-KLIGLER)

No.	Fermentation of Dulcite	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
23	+	+	+	—	—	B. columbarum	Kral
31	+	+	+	—	—	B. coli communis	P. D. & Co.
32	+	+	+	—	—	B. coli communis	Rosenau
34	+	+	+	—	—	B. coli communis	
35	+	+	+	—	—	B. coli communis	Feces
38	+	+	+	—	—	B. coli communis B.	Cystitis
57	+	+	+	—	—	B. aërogenes	Intestine of Dog
63	+	+	+	—	—	B. coli communis	Autopsy
73	+	+	+	—	—	B. acidi-lactici	Kral
79	+	+	+	—	—	B. choleræ	Kral
86	+	+	+	—	—	B. choleræ	Rahn

The saccharose-positive salicin-positive group (corresponding to *Bacillus aërogenes*) may again be further subdivided into 2 subgroups, one fermenting glycerin and not liquefying gelatin, the other liquefying gelatin and failing to ferment glycerin. Of these two properties, that

of glycerin fermentation is the more reliable. Of the 28 organisms falling in this group, 9 failed to ferment glycerin. Four of these gave about 1 c.c. of liquefaction in 30 days. Two others were sent to us as liquefying organisms, No. 1 as *Bacillus communior-rubra,* liquefying in 26 days, and No. 59 as *Bacillus cloacae,* but have apparently lost their liquefying power. This has been frequently observed among cloacae organisms and is another reason why liquefaction is unreliable as a test.

TABLE 7 (a)

SHOWING THE REACTIONS OF THE SACCHAROSE-NEGATIVE DULCITE-NEGATIVE GROUP (B. ACIDI-LACTICI-MACCONKEY)

No.	Fermentation of Salicin	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
34	+	+	+	—	—	B. coli communis	
55	—	+	+	—	—	B. acidi-lactici B.	Feces
56	—	+	+	—	—	B. acidi-lactici A.	Water
57	+	+	+	—	—	B. aërogenes	Intestine of dog
67	—	+	+	—	—	B. acidi-lactici	Milk
68	—	+	+	—	—	B. acidi-lactici	Milk
69	—	+	+	—	—	B. aërogenes	Milk
73	+	+	+	—	—	B. acidi-lactici	Kral

TABLE 7 (b)

SHOWING THE REACTIONS OF THE SACCHAROSE-NEGATIVE SALICIN-NEGATIVE GROUP (B. ACIDI-LACTICI-KLIGLER)

No.	Fermentation of Dulcite	Fermentation of Glycerin	Indol Production	Gelatin Liquefaction	V. and P. Reaction	Original Name	Source
29	+	+	+	—	—	B. coli communis	Novy
55	—	+	+	—	—	B. acidi-lactici B.	Feces
56	—	+	+	—	—	B. acidi-lactici A^2	Water
67	—	+	+	—	—	B. acidi-lactici	Milk
68	—	+	+	—	—	B. acidi-lactici	Milk
69	—	+	+	—	—	B. aërogenes	Milk

Another strain (No. 3) was sent to us as *Bacillus gasoformans,* a liquefying organism, which failed to liquefy, but did not attack glycerin. Seven out of the 9 glycerin non-fermenting organisms are thus liquefying forms. The other 2 which neither liquefy nor ferment glycerin came to us as paracoli and are aberrant in several other respects. It may therefore be tentatively concluded that the saccharose-positive

salicin-positive glycerin-negative forms are a distinct group which corresponds to the cloacae group. This group is so closely related to *Bacillus aërogenes* that it has often been impossible to differentiate them. The glycerin test should therefore prove of value in this connection. Table 8 gives a summary of the reactions of this group.

The 20 organisms which ferment dextrose but fail to ferment lactose fall into 2 groups, consisting of 5 gelatin liquefying proteus forms, and 15 non-liquefiers.

All the 5 proteus forms ferment dextrose and saccharose and fail to ferment any of the other sugars. Two of the 5 ferment glycerin and fail to produce indol, while the other 3 produce indol but fail to

TABLE 8

CHARACTERISTICS OF THE BACILLUS CLOACÆ

Organism	Dextrose	Lactose	Saccharose	Raffinose	Dulcite	Glycerin	Mannite	Salicin	Indol	Gelatin Liquefaction	V. and P. Reaction
40	+	+	+	+	+	−	+	+	−	+	+
50	+	+	+	+	+	−	+	+	−	+	+
51	+	+	+	+	+	−	+	+	−	+	+
59	+	+	+	+	+	−	+	+	−	−*	+
70		+	+	+	+	−	+	+	−	+*	+
1	+	+	+	+	+	−	+	+	−	−*	+
3	+	+	+	+	+	−	+	+	−	−*	+
89	+	+	+	+	+	−	+	+	−	−	+
91	+	+	+	+	−	−	+	+	−	−	+

* These came as liquefying organisms and have apparently lost that property under cultivation. None of the other characters has changed.

ferment glycerin. Four of these organisms (Nos. 11, 13, 14 and 17, see Table 9) came to us as *Bacillus vulgaris,* while No. 9 was sent to us as *Bacillus mirabilis.* From a study of 5 organisms it is, of course, impossible to determine whether the distinction is sufficiently important to separate them into 2 species. They may for the present be classed under *Bacillus vulgaris.* The distinctive characters of the species are summarized in Table 9.

The remaining dextrose-fermenters which failed to attack lactose are grouped in Table 10.

Table 10 points out that the organisms fall into two main divisions, one fermenting dulcite and the other failing to do so. Strains 24, 42 and 71 are aberrant and cannot be grouped with the others. Strains 24 and 71 correspond with their type, *Bacillus coscoroba* and *Bacillus*

capsulatus, respectively, except for their inability to ferment lactose. Strain 42 is interesting. It was sent to us as *Bacillus communis,* but repeatedly failed to ferment any of the sugars besides dextrose. In the hope that a reversion would be caused, the bacillus was passed

TABLE 9

CHARACTERS OF THE BACILLUS PROTEUS

Name of Organism	No. of Organism	Dextrose	Lactose	Saccharose	Dulcite	Salicin	Indol	Glycerin	Gelatin Liquefaction	V. and P. Reaction
B. mirabilis	9	+*	—	+	—	+	—	+	+	—
B. vulgaris	11	+	—	+	—	—	+	—	+	—
B. vulgaris	13	+	—	+	—	—	+	—	+	—
B. vulgaris	14	+	—	+	—	—	+	—	+	—
B. vulgaris	17	+	—	+	—	—	+	+	+	—

* The reactions of this group as given above agree with those recorded by Jordan in 1908.[15]

TABLE 10

SHOWING THE REACTIONS OF THE GELATIN-NEGATIVE LACTOSE-NEGATIVE DEXTROSE-POSITIVE GROUP

No. of Organism	Saccharose	Raffinose	Dulcite	Mannite	Glycerin	Salicin	Milk	Indol Production	V. and P Reaction	Original Name
24	+	+	+	+	+	+	+	—	—	B. coscoroba
42	—	—	—	+	—	—	—	+	—	B. communis
71	+	+	—	+	+	+	—	—	—	B. capsulatus
74	—	—	+	+	—	+	±	+	—	Paracolon b.
77	—	—	+	+	—	+	—	+	—	B. enteritidis
75	—	—	+	+	—	—	—	—	—	B. enteritidis
78	—	—	+	+	—	—	—	—	—	Paracolon b.
80	—	—	+	+	—	—	—	—	—	B. enteritidis
81	—	—	+	+	—	—	—	—	—	B. enteritidis
76	—	—	—	+	+	—	—	—	—	B. enteritidis
82	—	—	—	+	—	—	—	—	—	B. cholerasuis
83	+	+	—	+	—	+	—	—	—	B. cholerasuis
84	—	—	—	+	—	—	—	—	—	B. cholerasuis
85	—	—	—	+	—	—	—	—	—	B. cholerasuis
93	—	—	—	+	—	—	—	—	—	B. suis

successively through lactose broth for ten generations, with absolutely negative results. Of the other members of this group Strains 74 and 77 are identical, and though they fall in the dulcite-positive group they

15. *Jour. Hyg. Cambridge,* 1903, 3, p. 1.

differ from the others in fermenting salicin and producing indol, and should be classed by themselves as the paracolon bacilli. The remaining dulcite-fermenters are alike in their reactions and conform to *Bacillus enteritidis;* three of the four strains came to us under that name. The dulcite-negative forms were all (excepting Strain 76) obtained as *Bacillus cholera suis,* and with the exception of Strain 83, which seems to stand alone, all are characterized by the same fermentative properties.

No attempt was made to study the serum reactions of these organisms. Churchman and Michael[16] have, however, worked out the agglutination reactions of a few of our strains which belong to this group, and a comparison of their results with mine sheds some light on the importance of fermentation reactions when the proper sugars are used and quantitative data recorded. These authors used those organisms which came to us as *Bacillus enteritidis,* corresponding with Nos. 77, 76, 75, 80 and 81 (Table 10) of mine. A summary of their results is given in Table 11.

TABLE 11

SUMMARY OF AGGLUTINATION REACTIONS (CHURCHMAN AND MICHAEL)

Organism	Paratyphoid	Sera E. 75	E. 81	E. 76	E. 77
B. paratyphosus	+++	0	0	0	0
E. 75	0	+++	++	0	0
E. 80	0	+++	++	0	0
E. 81	0	++	+++	0	0
E. 76	0	0	0	+++	0
E. 77	0	0	0	0	+++

Table 11 indicates that Strains 75, 80 and 81 are identical, and that Strains 76 and 77 differ from them as well as from one another. This result corresponds exactly with my results with the fermentation reactions, Strain 76 differing from 77, and both reacting in an entirely different manner from the other apparently true enteritidis organisms. A further study of such correlations between fermentation and sera reactions would be of great interest and should prove valuable in definitely establishing the species relationship of this very complex and ill-defined group.

SUMMARY AND CONCLUSION

Eighty organisms generally classed under the colon group were subjected to a series of fermentative and other tests with a view of determining their natural grouping as based on biometric principles.

16. *Jour. Exper. Med.,* 1912, 16, p. 822.

Fifty-seven of these fell into the lactose-fermenting division; 20 did not ferment lactose, but fermented dextrose; 3 failed to ferment at all.

Acid production, as determined by titrating aliquot portions of the broth with phenolphthalein as an indicator, was found to be a more constant and a more reliable differential test than gas production, as ordinarily determined. The degree of initial acidity had no appreciable effect on the final acidity, which was quite constant, reaching its maximum on about the third day. The 57 lactose fermenters attacked mannite, glycerin, saccharose, salicin, raffinose, dulcite and inulin in the order named. Mannite, raffinose and inulin were considered to be of minor or doubtful classificatory importance. Saccharose divided the lactose group into two main subgroups.

On subdividing the saccharose groups, on the basis of dulcite and salicin fermentation respectively, it was found that the saccharose-salicin groups gave better correlation with indol production, Voges and Proskauer reaction and gelatin liquefaction than the saccharose-dulcite groups.

The saccharose-positive salicin-positive group (generally dulcite-negative) corresponds to *Bacillus aërogenes.*

The saccharose-positive salicin-negative group (generally dulcite-positive) corresponds to *Bacillus communior.*

The saccharose-negative salicin-positive group (generally dulcite-positive) corresponds to *Bacillus communis.*

The saccharose-negative salicin-negative group (generally dulcite-negative) corresponds to *Bacillus acidi-lactici.*

Glycerin was found to be of value in separating the cloacae forms from the aërogenes bacilli. Most of the saccharose-positive salicin-positive glycerin-negative group were gelatin liquefiers, indicating a reverse correlation between glycerin fermentation and gelatin liquefaction.

Of the dextrose fermenters, 5 of the organisms liquefied gelatin and fermented dextrose and saccharose, but failed to ferment any of the other sugars, with the exception of glycerin, which was fermented by 2 of the organisms. Of the other tests, all were negative with the exception of indol which was negative for the 2 glycerin-positive organisms and positive for the glycerin-negative bacteria. For the present all the 5 strains may be grouped under the *Bacillus vulgaris.*

The remaining dextrose fermenters which failed to ferment lactose and liquefy gelatin, were separated into three divisions: Those that

fermented dulcite and salicin and produced indol, apparently corresponding with the paracoli bacillus; *Bacillus enteritidis* group which fermented dulcite but neither attacked salicin nor produced indol, and *Bacillus cholerae suis* which fermented neither dulcite nor salicin and failed to produce indol. A comparison of the fermentation reactions with some agglutination tests made by Churchman bears out this grouping. The main results are summarized in Figure 3.

We realize, of course, that this classification is based on a relatively small number of organisms and can at best be considered only tentative. The results are, however, sufficiently suggestive and interesting to deserve recording, and to merit further investigations along the

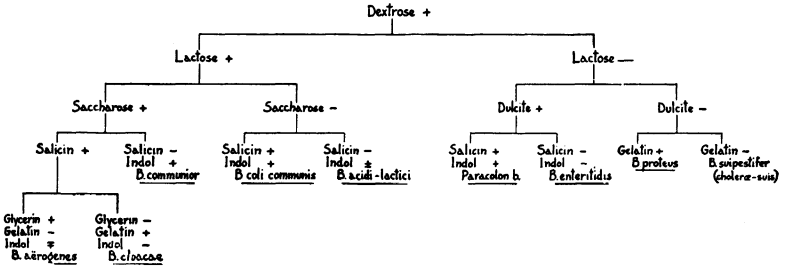

FIG. 3.—Diagrammatic representation of the salient characters of certain species of the colon-typhoid group.

lines indicated. From one viewpoint these results are valuable in that they are representative of a number of organisms of diverse origin, most of them, as far as we can tell, being isolated from distinct sources. In many studies a large number of strains are isolated from the same source, such as feces, water, etc. In these instances it is quite likely that about 50 per cent. of the strains from one source are but daughter cells of the same organism. A study of 100 forms does not, therefore, really represent the reactions of 100 distinct strains any more than 100 colonies, from a series of plates, sowed with a number of cultures of *Bacillus coli* would include as many different types. A study of the kind presented here is, therefore, of interest in that it gives the "mean" behavior of the groups, irrespective of the origin of the individual members belonging to it.